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AD NUMBER
AD809119
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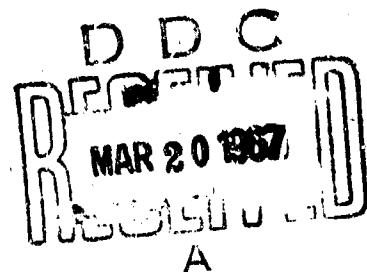
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TECHNICAL MANUSCRIPT 346

A UNIQUE DEFENSE MECHANISM
AGAINST ANTHRAX
DEMONSTRATED IN DWARF SWINE

Jerry S. Walker
Frederick Klein
Ralph E. Lincoln

FEBRUARY 1967



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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A UNIQUE DEFENSE MECHANISM AGAINST ANTHRAX
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Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A059

February 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ACKNOWLEDGMENT

We wish to extend our thanks to Morris A. Rhian, Albert L. Fernelius and Dean R. Hodges for their technical assistance.

A UNIQUE DEFENSE MECHANISM AGAINST ANTHRAX
DEMONSTRATED IN DWARF SWINE

ABSTRACT

A unique defense mechanism against Bacillus anthracis was demonstrated by the Pitman-Moore variety of dwarf swine. A large percentage of spores failed to germinate in vivo when massive doses were administered via either the respiratory or intraperitoneal route. Only spores with no apparent outgrowth of vegetative cells could be cultured from the blood. Therefore the "defense mechanism" enables the swine to prevent germination and outgrowth of B. anthracis spores in vivo.

In order to better understand the response of various animal species to infection with Bacillus anthracis, both susceptible and resistant species were studied. On the basis of reports in the literature^{1,2} miniature swine* were tested as one of the resistant species. Our report is restricted to this species. The dwarf or miniature swine of the Gulf region are believed to have originated from eight swine imported by Columbus in 1493 to the Spanish Indies.³ Since that time they have flourished in the swamps of the Gulf region, where they are called "or "woods" hogs.

Seventeen swine were challenged via the respiratory route in a 163-liter aerosol chamber.⁴ An aerosol with a particle diameter of 4.5 microns was generated with a Vaponefrin standard nebulizer.** Washed dormant spores of the Vollum Vlb strain were used.⁵ Twelve swine were sacrificed at planned intervals (Table 1). The remaining five swine, which had received inhalation doses of 10^8 to 10^7 spores, were observed for 30 days. They were rechallenged intraperitoneally (IP) with 1×10^{10} spores on the 10th day and with 2×10^{11} spores on the 12th day.

* Pitman-Moore Co., Zionsville, Indiana.

** Vaponefrin Co., Portland, Oregon.

TABLE 1. QUANTITATION OF SPORES AND VEGETATIVE CELLS IN SWINE SERIALY SACRIFICED

Swine No.	Time of Sacrifice, hr	Calculated Respiratory Dose ^a , 10 ⁷ org	Total B. anthracis Organisms in Tissues									
			Lungs		Spleen		Super P Node		Bronch. Node		Cerv. Node	
			10 ⁴ Org	Spores ^b /10 ⁴ Org	10 ⁴ Org	Spores ^b /10 ⁴ Org	10 ⁴ Org	Spores ^b /10 ⁴ Org	10 ⁴ Org	Spores ^b /10 ⁴ Org	10 ⁴ Org	Spores ^b /10 ⁴ Org
1	0 ^c /	9.0	900	7	890	10	1,000	45	1,700	19	1,000	16
2	1	2.9	25	40	10	77	26	28	50	26	19	67
3	2	9.9	11	100	cd/	ND ^e /	5	23	3	88	7	71
4	6	9.9	4	12	0.2	93	2	5	2	15	0.5	91
5	12	7.4	0.9	<100	2	90	2	46	10	15	22	60
6	18	12.0	C	ND	C	ND	C	ND	C	ND	C	ND
7	24	15.0	3	53	2	29	21	<100	2	75	0.7	41
8	36	12.0	C	ND	C	RD	C	ND	C	ND	C	RD
9	48	21.0	3	80	0	0	0.005	0	0	0	0	0
10	72	23.0	4	0.8	0	0	0	0	0	0	0	0
11	96	5.4	0.09	0	0	0	0	0	1	30	15	45
12	168	13.0	0.4	14	0	0	0	0	0	0	0	0

Calculated by Guyton's formula 8

a. Calculated by Guyton's formula.⁸

b. Per cent of heat-shockable spores.

c. Zero sacrifice time was approximately ½ hour after the end of the 15-minute exposure time.

d. Contaminated.

e. Not done.

The blood of the five swine was positive for spores (as determined by heat resistance) at the level of 100 to 200 spores per ml at 132 hours. Blood cultures were positive for spores obtained from one or more of these swine at different time periods up to 132 hours, but they were all negative after 132 hours. Vegetative cells were not detected in any case. All swine survived challenge, even those receiving three challenge doses, the last one of 2×10^{11} spores.

Tissue homogenates of the lungs, spleen, and suprapharyngeal, bronchial, and cervical lymph nodes were weighed, homogenized in a Servall multimix blender, diluted, and plated on tryptose glucose agar. Numbers of viable organisms per gram of tissue and percentage of spores (heat-resistant) are given in Table 1. A higher percentage than expected of the total organisms in the tissues were spores; vegetative cells were not detected in the blood. The failure of spores to germinate was also shown by Rosenwald, Jones, and Lincoln.* They found that only 33% of the spores germinated in blood leukocytes of 40- to 50-lb. Duroc swine during in vitro tests through 120 minutes. The large percentage of spores that failed to germinate with subsequent outgrowth contrasted with observations in other resistant and susceptible species,⁶ although F. Klein (unreported data) observed spores in the blood of NIH black rats for 30 days following IP challenge with 10^7 spores.

The inability to demonstrate vegetative cells in the blood of swine even after massive IP doses of spores suggests that swine, particularly dwarf swine, must have an unusual and effective "defensive mechanism" against B. anthracis. This observation on the blood was supported by (i) the large percentage of spores still not germinated in the organs tested even 24 hours after challenge, and (ii) the complete clearance of the lymph nodes and spleen after 48 hours. With the exception of the resistant rat, failure of spores to germinate was in contrast to germination in other species studied in our laboratory.

The results of our studies suggest that the natural environment and inbreeding have produced an unusually resistant variety of swine. The dwarf swine have demonstrated the domestic swine's mode of innate resistance to anthrax: that of preventing spore germination or outgrowth. The "defensive mechanism" possessed by swine that enables them to counteract the organism at this stage of infection is unknown. These results also indicate that breed and/or "line" susceptibility of swine should be investigated. Most likely, varied degrees of resistance exist among breeds as noted in other animal species.⁷

* Unpublished data.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of the Army Fort Detrick, Frederick, Maryland 21701		2a. REPORT SECURITY CLASSIFICATION Unclassified	
		2b. GROUP	
3. REPORT TITLE A UNIQUE DEFENSE MECHANISM AGAINST ANTHRAX DEMONSTRATED IN DWARF SWINE			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
5. AUTHOR(S) (Last name, first name, initial) Walker, Jerry S. Klein, Frederick (NMI) Lincoln, Ralph E.			
6. REPORT DATE February 1967		7a. TOTAL NO. OF PAGES 8	7b. NO. OF REFS 8
8a. CONTRACT OR GRANT NO. b. PROJECT NO. 1C522301A059 c. d.		9a. ORIGINATOR'S REPORT NUMBER(S) Technical Manuscript 346 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
10. AVAILABILITY/LIMITATION NOTICES Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Department of the Army Fort Detrick, Frederick, Maryland 21701	
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14. Key Words *Anthrax Inhalation *Immunity Intraperitoneal Miniature swine Infections Spores			

DD FORM 1473

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Security Classification